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Hepatic Neoplasms in the Mummichog *Fundulus heteroclitus* from a Creosote-contaminated Site[1]

Wolfgang K. Vogelbein,[2] John W. Fournie, Peter A. Van Veld, and Robert J. Huggett

The College of William and Mary, School of Marine Science, Virginia Institute of Marine Science, Gloucester Point, Virginia 23062 [W. K. V., P. A. V. V., R. J. H.], and United States Environmental Protection Agency, Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561 [J. W. F.]

ABSTRACT

High prevalences of idiopathic hepatic lesions were found in mummichog, *Fundulus heteroclitus*, from a site in the southern branch of the Elizabeth River, VA, contaminated with polycyclic aromatic hydrocarbons. Grossly visible hepatic lesions occurred in a total of 93% of the individuals from this site and 33% of these fish had hepatocellular carcinomas. Hepatic lesions were not detected in fish from two less contaminated sites. Lesions included foci of cellular alteration, hepatocellular adenoma, early and advanced hepatocellular carcinomas, and cholangiocellular proliferative lesions. Advanced carcinomas exhibited several distinct cellular patterns and some livers contained multiple neoplasms occupying up to 80% of the hepatic parenchyma. Sediments from the contaminated site contained extremely high concentrations (2200 mg/kg dry sediment) of polycyclic aromatic hydrocarbons, which are believed to originate from an adjacent wood treatment facility that has used creosote. Concentrations were 730- and 35-fold higher than those at the two other sites. These findings indicate a strong positive association between exposure to creosote-contaminated sediments and the high prevalence of hepatic neoplasms in a feral population of mummichog and support the putative role of polycyclic aromatic hydrocarbons in fish hepatocarcinogenesis. Additionally, they suggest that the mummichog may be a useful indicator of exposure to carcinogens in aquatic environments.

INTRODUCTION

Elevated prevalences of hepatic neoplasms have been reported in fishes from heavily contaminated coastal environments in the Puget Sound, WA (1–3), the lower Hudson River, NY (4), Boston Harbor, MA (5), and Los Angeles Harbor, CA (6). Similar associations have been observed in freshwater habitats (7–10). Xenobiotic chemical contaminants, particularly the PAHs,[3] are considered to be responsible for some of these "cancer epizootics" (11). This hypothesis is supported by laboratory exposure studies in which chemical carcinogens are used that generally elicit lesions similar to those observed in the field (12–14) and by studies demonstrating that fishes possess xenobiotic metabolizing enzyme systems capable of producing DNA-reactive carcinogenic metabolites (15, 16).

The Elizabeth River, VA, is a highly industrialized waterway that divides the cities of Norfolk and Portsmouth at its confluence with the lower Chesapeake Bay. This river receives wastes from naval and commercial shipyards, petrochemical storage and processing facilities, heavy industry, municipal sewage treatment plants, and urban and agricultural runoff (17). Portions of the Elizabeth River are highly contaminated with PAHs derived primarily from creosote (18–20). Several recent studies suggest that the occurrence of various pathological abnormalities (21–24) in Elizabeth River fish may be linked with exposure to PAH-contaminated sediments. However, hepatic neoplasms were not detected in those surveys. The present study was designed to examine a small nonmigratory estuarine fish from a heavily PAH-contaminated site in the Elizabeth River in an attempt to further link the occurrence of hepatic neoplasms in fish to PAH contamination.

We report here a very high prevalence of liver cancer in a population of mummichog, *Fundulus heteroclitus* from a creosote-contaminated site in the Elizabeth River's Southern Branch. Comparative lesion prevalences in mummichog from 3 study sites as well as gross and histological features of putative preneoplastic and neoplastic hepatocellular lesions in fish from the contaminated site are described.

MATERIALS AND METHODS

Fish Collection and Treatment. Adult mummichog were collected in baited minnow traps during August 1989 from three different localities (Fig. 1). Station 1, a relatively uncontaminated reference site, was located in Wilson's Creek, a tributary of the Ware River, Gloucester, VA. Station 2, a moderately contaminated site, was located in the mouth of Scuffeltown Creek, at the east end of the Jordan Toll Bridge; and Station 3 was located directly across the Elizabeth River from Station 2 (approximately 600 m away), in a small tidal creek adjacent to Atlantic Wood Industries, Inc., an active wood treatment facility. Only adult fish (total length, ≥75 mm) were selected and were transported live to the laboratory. Fish were maintained in an open circulating system and necropsied within 3–4 days after capture.

For histopathological examination, individual fish were anesthetized with tricaine methanesulfonate (MS-222; Sigma Chemical Co.), examined grossly, sexed, measured (standard and total lengths), and weighed. Visceral organs were dissected and examined. The liver was carefully separated from the other organs, weighed, photographed, and cut with a razor blade into several strips 2–5 mm thick. Liver tissue was fixed in Bouin's solution for 24–48 h and processed by routine methods for paraffin histology (25). The 2–5-mm-thick tissue strips comprising individual livers were embedded in one paraffin block to permit simultaneous sectioning at multiple levels within each liver. Sections were cut at 5 μm and stained routinely with Harris' hematoxylin and eosin Y (25). A total of 105 fish were evaluated histologically.

Histological slides of representative cases, including all lesions illustrated in this report, have been deposited in the Registry of Tumors in Lower Animals, Smithsonian Institution, Washington, DC (RTLA 4947-4968).

Sediment Collection and PAH Analysis. Sediment samples for PAH analysis were collected with a Smith-MacIntyre grab. The upper 2 cm of sediment were removed with a metal scoop, transferred to clean glass jars, and frozen until analyzed.

Analytical procedures used for sediment-PAH analysis have been described previously (18). Briefly, samples were thawed, homogenized, and lyophilized. The dried sediment samples received an internal standard consisting of 1,1′-binaphthyl and perinaphthenone and were then extracted for 48 h in glass Soxhlets. Coextracted high molecular weight biogenic materials were removed by gel permeation chromatography. Aromatic fractions were isolated from the partially purified extract by silica gel chromatography. The fractions containing aromatic compounds were analyzed by capillary gas chromatography with flame ionization detection. Quantitation was accomplished by comparison of the chromatographic peak areas present in samples with that of the internal standard.

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[2] To whom requests for reprints should be addressed, at the College of William and Mary, School of Marine Science, Virginia Institute of Marine Science, Gloucester Point, VA 23062.

[3] The abbreviation used is: PAHs, polycyclic aromatic hydrocarbons.

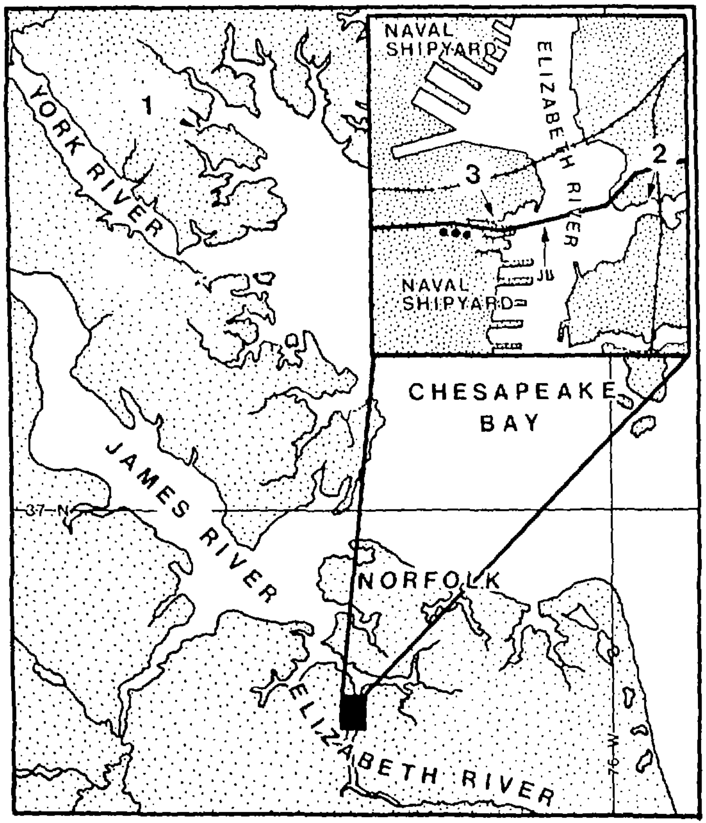

Fig. 1. Sampling station location map depicting three sites where mummichog, *F. heteroclitus*, were collected. Station 1, Wilson's Creek reference site; Station 2 (*inset*), Scuffeltown Creek site; and Station 3 (*inset*), creosote-contaminated site adjacent to Atlantic Wood Industries, Inc. (●●●). *JB*, Jordan Toll Bridge.

Table 1 *Lesion prevalences for F. heteroclitus and sediment PAH concentrations from three sampling localities in Chesapeake Bay*

	No. of fish with lesions (lesion prevalence)		
Lesion types	Station 1 ($N = 15$)[a]	Station 2 ($N = 30$)	Station 3 ($N = 60$)
Foci of cellular alteration	0	0	44 (73.3)[b]
Cholangiolar proliferation	0	0	7 (11.7)
Hepatocellular adenoma	0	0	1 (1.7)
Early hepatocellular carcinoma	0	0	8 (13.3)
Advanced hepatocellular carcinoma	0	0	12 (20.0)
Total hepatocellular neoplasms	0	0	21 (35.0)
Total resolved sediment PAHs (mg/kg dry wt)	3	61	2200

[a] *N*, number of fish examined histologically.
[b] Numbers in parentheses, percentage.

RESULTS

Sediment Chemistry. Results of the sediment PAH analyses are summarized in Table 1. Total resolved sediment PAH concentrations were lowest at the Wilson Creek reference site (Station 1). They were 20-fold higher at the Scuffeltown Creek site (Station 2) and 730-fold higher at the creosote-contaminated site (Station 3).

Lesion Prevalence. Lesion prevalences for 105 mummichog from the three sampling stations are summarized in Table 1. Preneoplastic and neoplastic lesions were observed only in fish from Station 3, the highly contaminated site. The majority of these fish also exhibited moderate to severe hepatocellular lipoidosis and ceroidosis. The number and size of hepatic macrophage aggregates were greatly elevated in these fish. Mummichog from Station 2 exhibited no neoplastic lesions; however, many of these fish exhibited moderate to severe lipoidosis and diffuse hepatocellular ceroidosis with an associated increase in the size and number of macrophage aggregates. Livers of fish from Station 1, the relatively uncontaminated reference site, were histologically normal.

Gross Liver Pathology. The livers of fish from Stations 1 and 2 appeared normal by gross examination. The liver capsule was smooth, liver lobes were undefined, and livers varied in color from pale tan to reddish brown. They were texturally homogeneous and without discrete focal lesions (Fig. 2*a*). Livers of fish from Station 3, however, presented a spectrum of pathological changes. Grossly visible alterations were observed in 93% (56/60) of the livers and included: (*a*) pronounced hepatomegaly; (*b*) mottling of the hepatic parenchyma; (*c*) small (0.2–1.0 mm in diameter), pale tan subcapsular focal lesions (Fig. 2*b*); (*d*) discrete raised nodular lesions of variable color, shape, and size; (*e*) cystic lesions, raised or not, containing clear, green, or reddish brown fluid; and (*f*) discrete, relatively large (0.5–1.5 cm in diameter) tumors within the hepatic parenchyma (Fig. 2*c*). In most cases these tumors were pearly white, with a well-defined surface vasculature. Macrophage aggregates were elevated in number and size in tumorous livers (Fig. 2*c*). Lesion types cooccurred in various combinations within individual livers.

Normal Liver Structure. Liver structure of mummichog from Stations 1 and 2 was similar to that described for other teleosts (26, 27) and consisted of anastomosing cords (muralia) of hepatic parenchymal cells lined on both sides by the hepatic sinusoids (Fig. 2*d*). Muralia were generally 1 or 2 cells thick. Hepatocytes were monomorphic, with heterochromatic nuclei of a uniform shape and size. Nuclei were spherical and generally had one prominent central nucleolus. Staining of the hepatocyte cytoplasm varied considerably among fish but was always homogeneous within individual livers. There was considerable variation in the degree of cytoplasmic vacuolization among fish. Macrophage aggregates were generally small, occurred in low numbers, and were mostly associated with the pancreatic acini but also occurred free within the hepatic parenchyma.

Foci of Cellular Alteration. Three distinct types of hepatic parenchymal lesions were diagnosed as foci of cellular alteration. These lesions were variable in shape, ranged from 0.1 to 3.5 mm in diameter, and exhibited minimal deviation from normal hepatocellular architecture (Fig. 3*a*). Constituent hepatocytes blended into the surrounding normal parenchyma with little or no evidence of compression or invasiveness (Fig. 3*b*). Bile ducts, exocrine pancreatic tissue, and macrophage aggregates were generally excluded from these altered foci. Intracellular storage products such as lipid, glycogen, and ceroid often were greatly reduced or absent. Mitoses were rarely observed.

1. Eosinophilic foci included two morphological subsets as follows. (*a*) A numerically dominant subset within this category was composed of altered hepatocytes that ranged in size from somewhat smaller than normal to somewhat hypertrophied (Fig. 3*b*). The cytoplasm was eosinophilic with a ground glass or fibrillar consistency. In some foci nuclei were intensely hyperchromatic and pleomorphic. Nucleoli were prominent, single, and centric or eccentric. (*b*) A second less common subset of eosinophilic foci consisted of altered hepatocytes that were greatly hypertrophied. The border between focus and normal parenchyma was irregular, especially in the large foci (Fig. 3*c*). Hepatocytes exhibited a ground-glass cytoplasm and often contained prominent hyaline inclusions (Fig. 3*d*). Nuclei were slightly enlarged.

2. Basophilic foci were generally round or oval (Fig. 3*e*). Constituent hepatocytes were normal in size and often con-

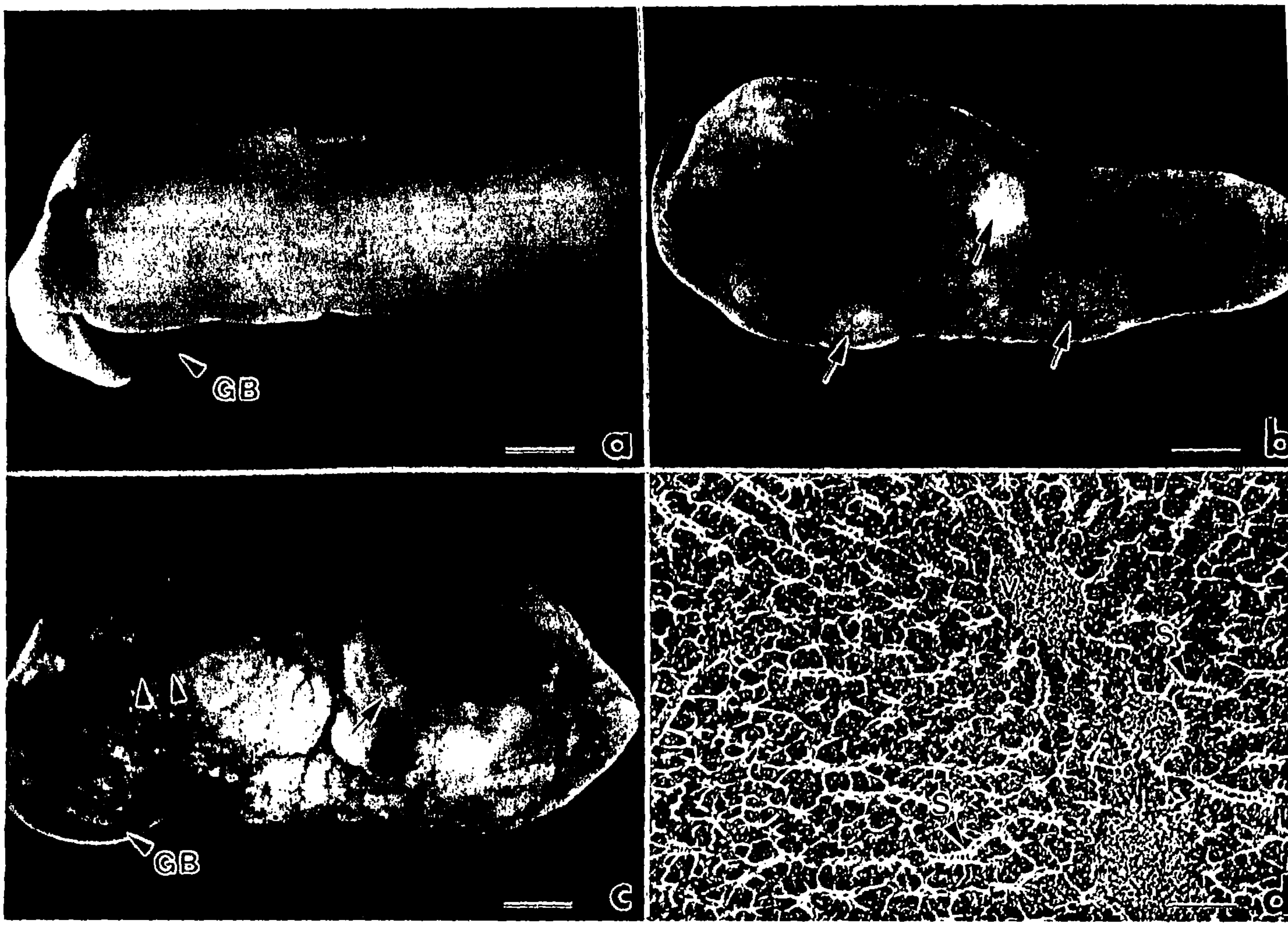

Fig. 2. Gross liver pathology and normal liver histology of the mummichog from three Chesapeake Bay collection sites. *a*, liver of fish from reference site (Station 1); *GB*, gallbladder; *bar*, 2.6 mm. *b*, liver of fish from Station 3 exhibiting multiple pale tan focal lesions (*arrows*); *bar*, 2.6 mm. *c*, liver of fish from Station 3 exhibiting multiple focal lesions and one 8.0-mm-diameter hepatocellular carcinoma (*arrow*). *Arrowheads*, macrophage aggregates; *GB*, gallbladder; *bar*, 2.6 mm. *d*, normal hepatocellular architecture from reference site fish (Station 1). *S*, hepatic sinusoids; *V*, hepatic vein; *bar*, 60 μm. Harris' hematoxylin and eosin.

tained moderate amounts of lipid, hyaline material, or ceroid but exhibited a hyperbasophilic cytoplasm. Nuclei were normal or slightly enlarged, were euchromatic, and contained a prominent nucleolus.

3. Clear cell foci varied considerably in shape and consisted of extensively vacuolated hepatocytes. Cells were normal cytologically except for excessive lipid or glycogen reserves.

Some foci of cellular alteration contained groups of more radically altered hepatocytes within them (Fig. 3*f*). The cellular architecture of these "foci within foci" was often more poorly differentiated and resembled the morphology of hepatocellular carcinomas (Fig. 4*a*). Furthermore, cells of foci within foci exhibited greater cellular and nuclear pleomorphism and intracellular stores of lipid or glycogen were greatly reduced. They were observed in all three types of altered foci.

Hepatocellular Adenoma. A single lesion was diagnosed as hepatocellular adenoma. The adenoma was spherical, was 4.2 mm in diameter, and exhibited a sharp border with slight compression of the adjacent parenchyma (Fig. 4*b*). Exocrine pancreatic acini, bile ducts, and macrophage aggregates were absent from the lesion. The cellular architecture was atypical, with hepatic sinusoids considerably dilated and arranged perpendicular with respect to sinusoids in the adjacent parenchyma (Fig. 4*c*). Hepatocytes comprising the adenoma were hypertrophied with an eosinophilic ground-glass cytoplasm and enlarged nuclei that were slightly pleomorphic. Mitotic activity was slightly elevated. Nonneoplastic hepatocytes at the lesion border contained large quantities of lipid and ceroid.

Early Hepatocellular Carcinoma. Lesions diagnosed as early carcinomas were small and compact (0.2–0.8 mm in diameter). They apparently arose *de novo* within the nonneoplastic parenchyma (Fig. 4, *d* and *e*) or within foci of cellular alteration (Figs. 3*f* and 4*a*). These lesions exhibited a disorganized cellular architecture and several were highly anaplastic. Borders were often very irregular, with neoplastic cells invading the adjacent hepatic parenchyma (Fig. 4, *d–f*). The neoplastic hepatocytes were pleomorphic and disorganized. The cytoplasm was eosinophilic or pale blue-gray and often fibrillar. Mitoses were generally rare. Nuclei were hypertrophied and euchromatic with prominent, often multiple nucleoli. Some lesions exhibited tumor giant cell formation (Fig. 4*f*).

Advanced Hepatocellular Carcinoma. Advanced carcinomas varied greatly in shape and ranged from 0.5 to 1.5 cm in diameter. Several livers contained multiple neoplasms occupying up to 80% of the hepatic parenchyma. Carcinomas ranged from moderately well-differentiated to poorly differentiated lesions exhibiting extreme anaplasia. Some larger lesions varied regionally in their degree of differentiation.

Well-differentiated carcinomas were sharply demarcated from the normal parenchyma, often by a zone of hepatocytes

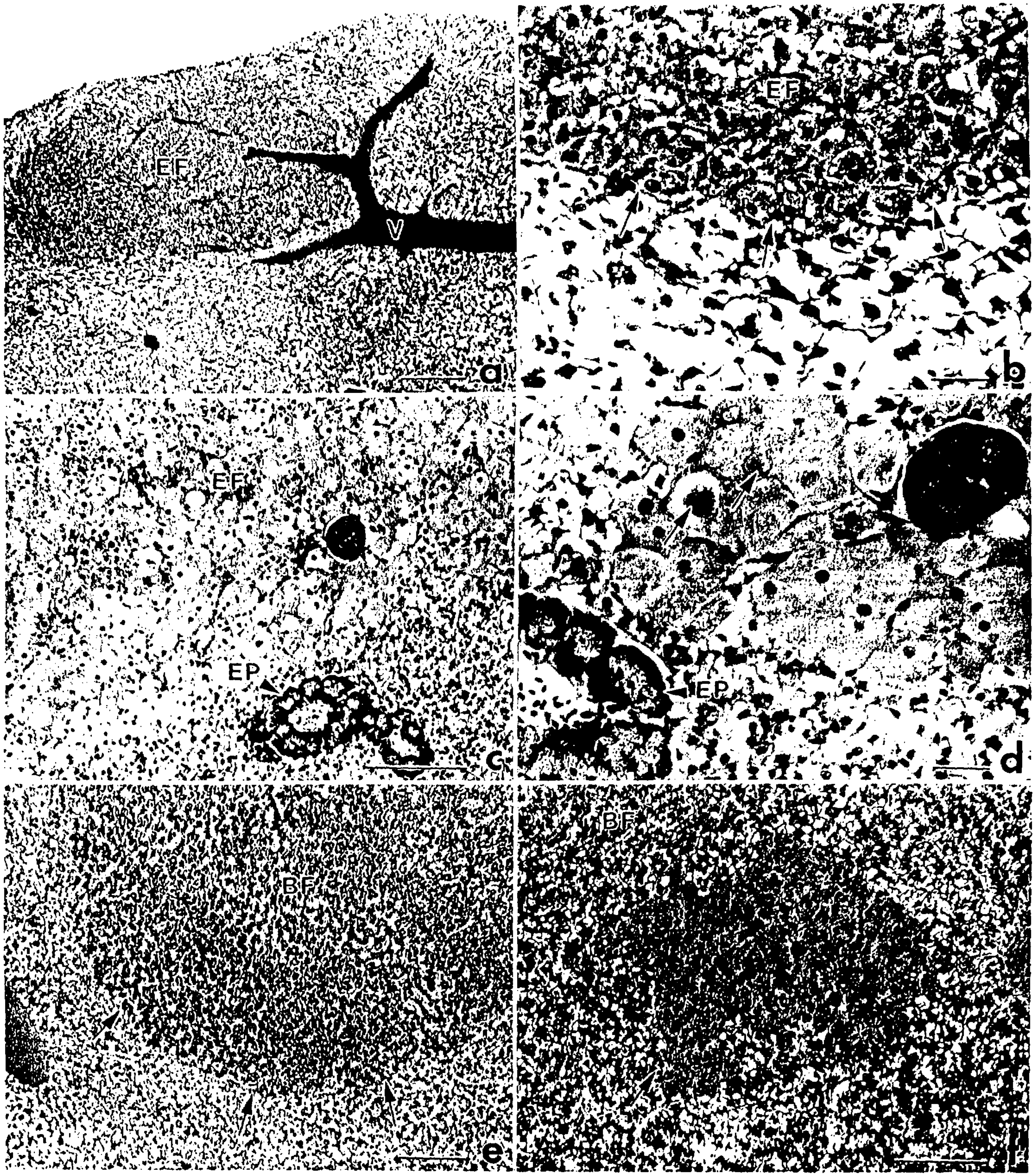

Fig. 3. Foci of cellular alteration in mummichog from a creosote-contaminated site. *a*, eosinophilic focus (*EF*); *V*, hepatic vein; *bar*, 250 μm. *b*, eosinophilic focus (*EF*) illustrating border with normal hepatic parenchyma (*arrows*); *bar*, 22.6 μm. *c*, eosinophilic focus, hypertrophied cell variant (*EF*). *EP*, exocrine pancreas; *bar*, 100 μm. *d*, eosinophilic focus, hypertrophied cell variant; *arrows*, hyaline bodies; *EP*, exocrine pancreas; *bar*, 22.6 μm. *e*, basophilic focus (*BF*). *Arrows*, border with normal hepatic parenchyma; *bar*, 250 μm. *f*, group of more radically altered hepatocytes (*arrow*) within a basophilic focus (*BF*) ("focus within a focus"); *bar*, 100 μm. Harris' hematoxylin and eosin.

containing increased amounts of lipid (Fig. 5*a*). Some lesions were locally invasive (Fig. 5*a*) and exhibited a trabecular growth pattern; however, the muralial architecture was generally still recognizable (Fig. 5*b*). Hepatocyte nuclei were slightly pleomorphic, greatly hypertrophied, and euchromatic and contained prominent nucleoli. The nuclear:cytoplasmic ratio was gener-

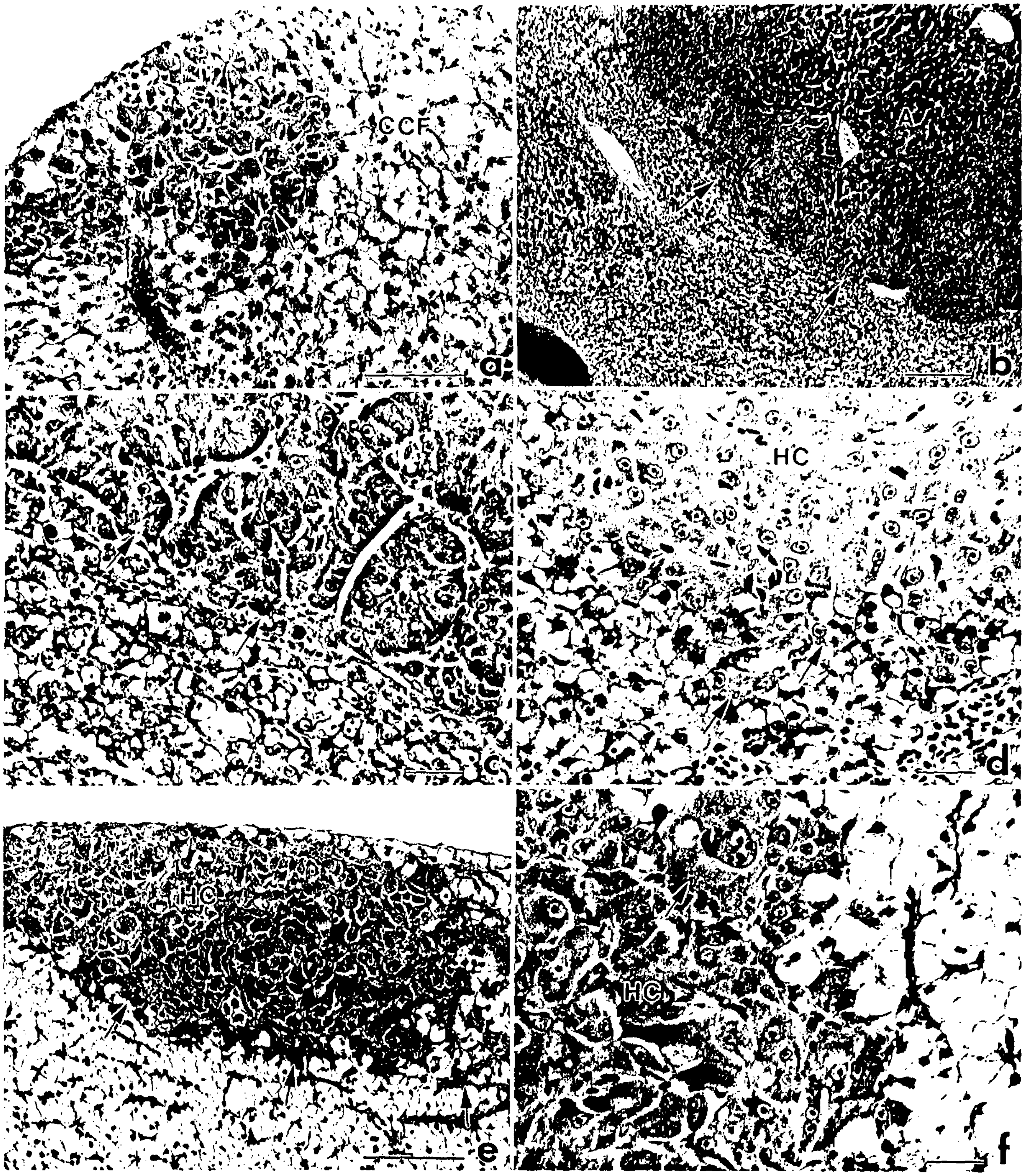

Fig. 4. Hepatoproliferative lesions in mummichog from a creosote-contaminated site. *a*, focus of more radically altered hepatocytes (*arrow*) within a clear cell focus (*CCF*); *bar*, 100 μm. *b*, hepatocellular adenoma (*A*). *Arrows*, border between lesion and adjacent nonneoplastic parenchyma; *bar*, 250 μm. *c*, higher magnification of adenoma (*A*) illustrated in *b*. *Arrows*, border with slightly compressed adjacent hepatic parenchyma; *bar*, 22.6 μm. *d*, early hepatocellular carcinoma (*HC*). *Arrows*, neoplastic hepatocytes invading adjacent nonneoplastic parenchyma; *bar*, 22.6 μm. *e*, early hepatocellular carcinoma (*HC*). *Arrows*, irregular, locally invasive border of neoplasm; *bar*, 100 μm. *f*, higher magnification of carcinoma (*HC*) illustrated in *e*. *Arrow*, tumor giant cell; *bar*, 22.6 μm. Harris' hematoxylin and eosin.

ally elevated and mitoses were often abundant. The cytoplasm of neoplastic cells was hyperbasophilic or eosinophilic, sometimes with a prominent fibrillar texture. Lipid and glycogen stores were greatly reduced or absent.

Less well-differentiated neoplasms were generally large, locally invasive, and compressive. Several contained large fluid-

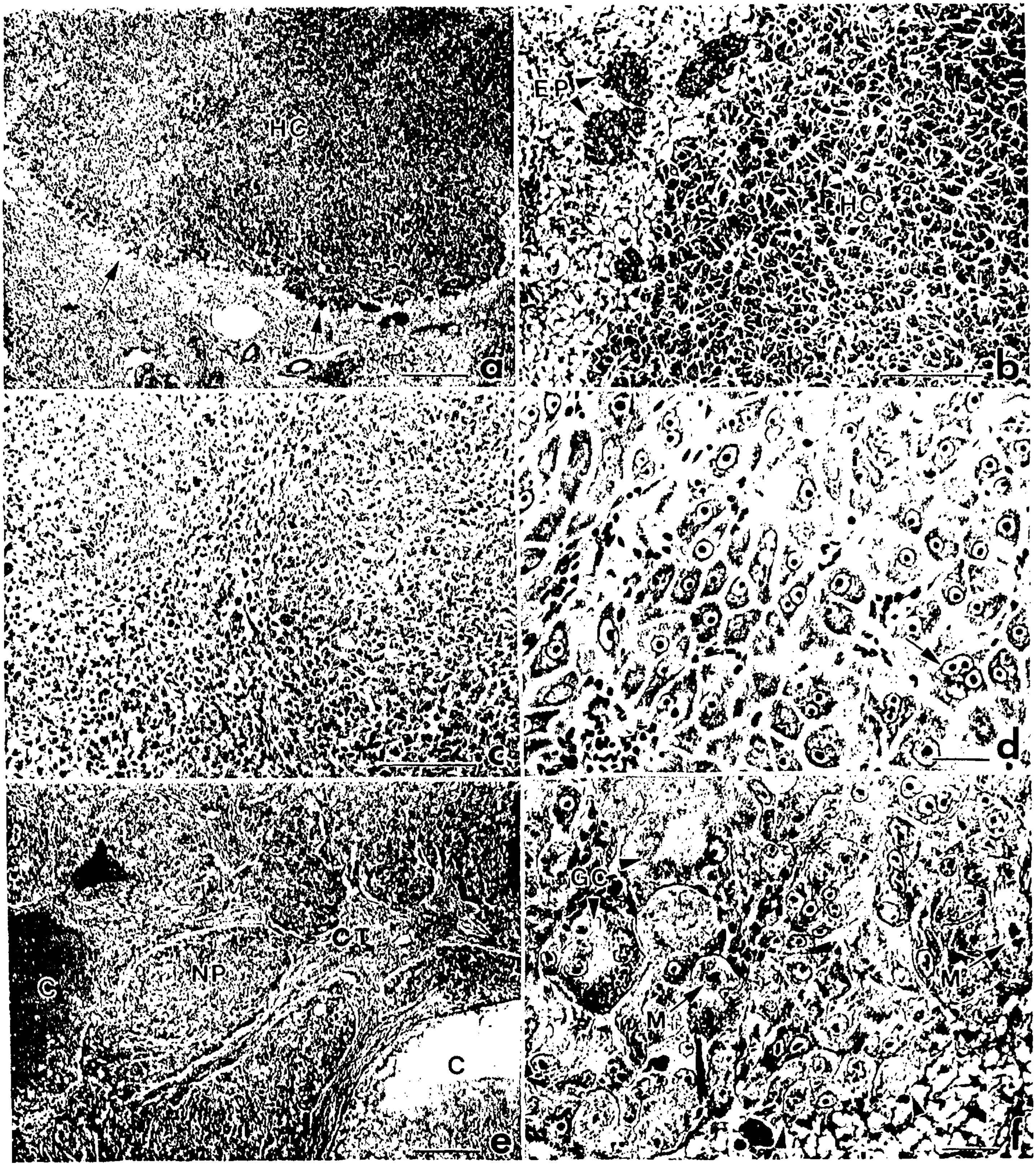

Fig. 5. Hepatocellular carcinomas in mummichog from a creosote-contaminated site. *a*, well-differentiated hepatocellular carcinoma (*HC*). *Arrows*, irregular, locally invasive border and lipid-rich zone of nonneoplastic hepatocytes; *bar*, 250 μm. *b*, higher magnification of carcinoma (*HC*) illustrated in *a*; *EP*, exocrine pancreas; *bar*, 100 μm. *c*, less well-differentiated hepatocellular carcinoma. Note extreme cellular pleomorphism; *bar*, 100 μm. *d*, less well-differentiated hepatocellular carcinoma comprised of epithelial-appearing tumor cells; *arrow*, multinucleated neoplastic hepatocyte; *bar*, 22.6 μm. *e*, poorly differentiated hepatocellular carcinoma exhibiting multilobulated histological pattern with prominent stromal component (*CT*), fluid- and blood-filled cystic spaces (*C*), and lobules of neoplastic parenchymal cells (*NP*); *bar*, 250 μm. *f*, poorly differentiated anaplastic hepatocellular carcinoma exhibiting epithelial-appearing constituent cells. *M*, mitotic figure; *GC*, multinucleate tumor giant cell; *Arrowheads*, border of neoplasm; *bar*, 22.6 μm. Harris' hematoxylin and eosin.

or blood-filled cystic spaces. They varied regionally in their degree of differentiation with some exhibiting extensive cellular pleomorphism (Fig. 5*c*) and others composed of better differentiated, epithelial-appearing tumor cells (Fig. 5*d*). In both cases the normal muralial architecture was disrupted. Constituent cells of all less well-differentiated neoplasms were pleomorphic and lacked polarity. Many cells were hypertrophied and some were multinucleate. The hepatocyte cytoplasm in most of these tumors was eosinophilic and slightly fibrillar. Nuclear pleomorphism was slight to moderate (Fig. 5*d*). Nuclei were greatly enlarged and euchromatic and contained prominent, often multiple nucleoli. Mitotic activity varied from slight to moderate.

Two fish had very large, poorly differentiated hepatic tumors. One neoplasm exhibited a pleomorphic giant cell pattern and consisted of lobules and masses of highly atypical epithelial cells. Portions of this tumor exhibited a prominent stroma, with connective tissue septa segregating lobules of neoplastic cells (Fig. 5*e*), while other regions lacked a prominent connective tissue component. The tumor contained several large blood- and fluid-filled cystic spaces (Fig. 5*e*) that exhibited a peripheral fibrosis and leukocytic infiltration. Constituent neoplastic cells exhibited a high degree of anaplasia and a prominent epithelial appearance (Fig. 5*f*). Mitoses were very abundant and often atypical and bizarre. Multinucleate tumor giant cells were abundant (Fig. 5*f*). Some of these cells exhibited a central zone of eosinophilia and a peripheral border of basophilic fibrillar material (Fig. 5*f*).

The other fish had a large, poorly differentiated, highly anaplastic hepatocellular carcinoma within which arose a hepatoblastoma-like neoplasm. This portion of the tumor appeared as a well-circumscribed, hyperbasophilic mass that was 2.5 x 3.0 mm in diameter. It consisted of small, undifferentiated embryonal cells with a very high nuclear:cytoplasmic ratio (Fig. 6*a*). Mitotic activity was extremely high with up to 75 mitoses per 40× microscopic field (Fig. 6*b*). Constituent cells were small with oval or elongated hyperchromatic nuclei and a thin rim of pale cytoplasm. Cell borders were indistinct. Portions of this lesion contained small vascular channels and in some sections, arrangement of the cells bordering these blood vessels suggested rosette formation (Fig. 6*a*). The associated carcinoma exhibited extreme anaplasia. Neoplastic cells were small with indistinct cell borders and contained small, pleomorphic, elongated, heterochromatic nuclei. The cellular architecture was

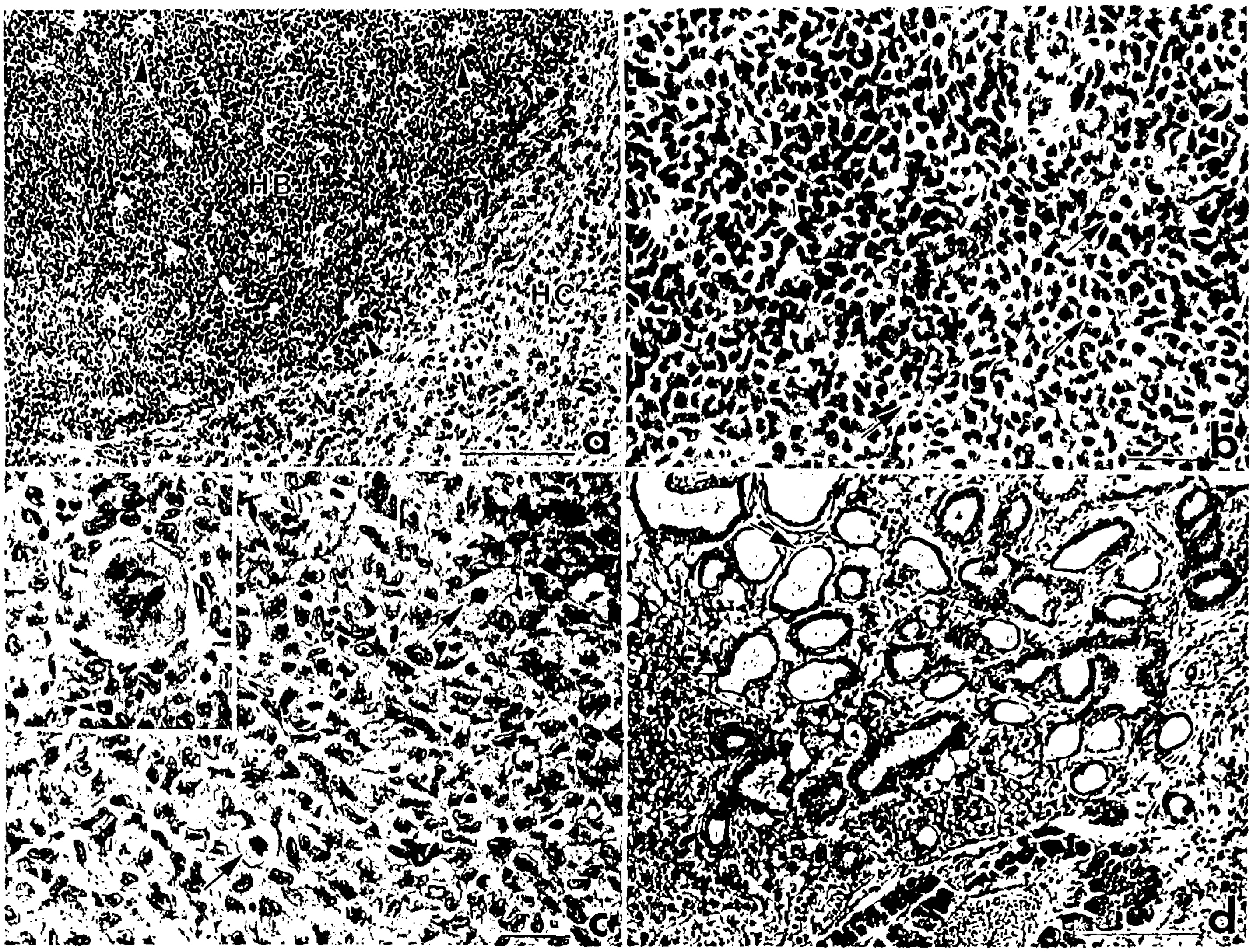

Fig. 6. Hepatoproliferative lesions in mummichog from a creosote-contaminated site. *a*, undifferentiated hepatoblastoma-like neoplasm (*HB*) within hepatocellular carcinoma (*HC*). *Arrowheads*, rosette like formations surrounding blood vessels; *bar*, 100 μm. *b*, higher magnification of hepatoblastoma like lesion in *a* exhibiting hypercellularity and high mitotic activity (*arrows*); *bar*, 22.6 μm. *c*, anaplastic cellular pattern of hepatocellular carcinoma at periphery of hepatoblastoma. *Arrows*, mitotic figures; *inset*, tumor giant cell; *bar*, 22.6 μm for figure and *inset*. *d*, focal bile duct proliferation; *bar*, 100 μm. Harris' hematoxylin and eosin.

haphazard and mitotic activity was high (Fig. 6*c*). Multinucleate tumor giant cells (Fig. 6*c*, *inset*) were prominent. Some of these features are characteristic of a neoplasm of histiocytic origin. However, major portions of this neoplasm exhibit an epithelial morphology and we believe that this lesion is a poorly differentiated hepatocellular carcinoma.

Cholangiocellular Proliferative Lesions. Seven of the 60 fish from Station 3 (11.7%) exhibited proliferative bile duct lesions (Fig. 6*d*). These lesions were small and composed of hyperplastic bile ducts, or large bile-filled cysts lined by normal to slightly atypical biliary epithelium (not illustrated). Epithelial cells resembled those comprising normal bile ducts in their tinctorial properties and nuclear morphology. All lesions exhibited prominent interstitial fibroplasia and leukocytic infiltration, and in some the epithelium exhibited squamous metaplasia (Fig. 6*d*).

DISCUSSION

Histopathological and chemical analyses in this study suggest a positive relationship between chronic exposure to PAH-contaminated sediments and the occurrence of hepatic neoplasms in a population of Elizabeth River mummichog. Putative preneoplastic and neoplastic hepatic lesions occurred in 93% of the individuals from a highly PAH-contaminated site adjacent to a wood-creosoting facility. However, these lesions were not detected in fish from two less-contaminated sites. The prevalence of hepatocellular carcinomas (33%) in Elizabeth River mummichog is perhaps the highest ever reported in a feral fish population inhabiting a PAH-contaminated environment (11).

Sediment PAH concentrations in the Elizabeth River are among the highest found anywhere in the United States (18, 19). Maxima occur in the Elizabeth River's Southern Branch and coincide with the locations of several abandoned wood treatment facilities and the active plant adjacent to our study site. Part of the PAH contamination has been attributed to several massive creosote spills that occurred at these facilities during the 1960s (18, 20). Polycyclic aromatic hydrocarbon concentrations in sediments from the tidal creek adjacent to an active wood treatment facility (Station 3, this study) were similar in magnitude to those reported from another nearshore station adjacent to a now-abandoned wood treatment plant (28). However, PAH concentrations at these inshore stations were at least 6 times greater than those in sediments taken from midchannel (18, 19). This lends support to earlier findings implicating these facilities as one source of the PAH contamination in this river (18, 20).

Coal tar creosote is an environmentally persistent chemical mixture that consists of approximately 85% PAHs, 10% phenolic compounds, and 5% heterocyclic compounds (29). It has been demonstrated to be mutagenic in the Ames test (30) and induces skin neoplasms in rodents (31). Experimental studies designed to test the potential carcinogenicity of creosote in fishes have not been conducted. However, a high prevalence of hepatic neoplasms in the English sole, *Parophrys vetulus*, from a creosote-contaminated harbor in Puget Sound, WA, was linked with exposure of the fish to certain constituents of creosote (32). Eagle Harbor sediments contained particularly high concentrations of aromatic hydrocarbons (maximum of 120,000 ng/g dry weight) and lesser concentrations of the nitrogen heterocycles and chlorinated hydrocarbons. All three classes of compounds are known to include potent carcinogens (33). Many of these sediment-associated chemicals, notably the PAHs, were bioaccumulated by Eagle Harbor English sole, with high PAH concentrations in stomach contents suggesting that dietary sources were an important route of uptake. Moreover, high concentrations of PAH metabolites in the bile of Eagle Harbor sole demonstrated that the fish accumulated constituents of creosote and actively metabolized them. (32).

In addition to field observations, laboratory studies have demonstrated that certain PAHs are carcinogenic in fishes. Development of hepatic and extrahepatic neoplasms in the guppy occurred following four 6-h aqueous exposures to 7,12-dimethylbenz(*a*)anthracene at 1-week intervals (34). Water-borne exposures to 7,12-dimethylbenz(*a*)anthracene have also been shown to induce hepatic neoplasms in *Poeciliopsis lucida* and *Poeciliopsis monacha* (35) and a single s.c. injection of this potent carcinogen was shown to elevate the incidence of melanotic neoplasms in the skin of the croaker, *Nibea mitsukurii* (36). Benzo(*a*)pyrene, another PAH, induced hepatic neoplasms in rainbow trout following dietary exposure, i.p. injection (37), and embryo microinjection (38). Additionally, the hepatocarcinogenic effects of water-borne exposure to benzo(*a*)pyrene in two small fish species were recently demonstrated for the first time (39).

Diagnoses for the hepatic lesions in Elizabeth River mummichog were based on histological criteria developed for the carcinogen-induced hepatoproliferative lesions of laboratory rodents (40–42). Foci of cellular alteration in Elizabeth River mummichog morphologically resembled those described in other feral (3) and laboratory fishes (13, 14, 28, 34, 39) exposed to hepatocarcinogens. Although we have little information regarding the biological behavior of these lesions, our histological data suggest that the three types of altered foci should be considered preneoplastic in the mummichog. The occurrence of more highly atypical foci within eosinophilic, basophilic, and clear-cell foci suggests that some of these lesions progress to carcinoma. In contrast, eosinophilic foci of the rainbow trout, *Salmo gairdneri*, are not considered to be of significance in the development of hepatic neoplasms (14). In that species, this lesion is often infiltrated by leukocytes presumed to represent part of a cellular immune response, and transition to the basophilic focus or carcinoma has not been observed. The eosinophilic focus of the English sole, *Parophrys vetulus*, on the other hand, is usually not infiltrated by host leukocytes and lesion cooccurrence analyses (3) suggest a strong positive correlation between this lesion and basophilic foci, adenomas, and hepatocellular carcinomas. Whereas eosinophilic and basophilic foci in this species are considered to be preneoplastic (3), the basophilic focus of the rainbow trout is thought to be a small, noncompressing carcinoma rather than a preneoplastic lesion (14).

Several attributes suggest that the mummichog may be an appropriate sentinel of exposure to hepatocarcinogens in aquatic environments. This small cyprinodontid teleost inhabits shallow estuarine coves, inlets, and tidal creeks along much of the eastern seaboard of the United States (43). Despite its broad geographic distribution, this species is considered nonmigratory and exhibits a restricted summer home range of 30–40 m (44), with limited movements occurring only during the autumn and winter (45). A restricted subpopulation, such as the one inhabiting the PAH-contaminated site (Station 3) in the Elizabeth River, may thus reflect the "health" of its immediate environment. Because of the mummichog's apparent tumorigenic sensitivity, its nonmigratory behavior, and its wide geographic distribution, this killifish lends itself as perhaps no other small fish species could to serve as a bioindicator of chemical carcinogens in estuarine environments. Additionally, the relatively small size of this fish and its broad tolerance to fluctuations in environmental conditions make it an ideal subject for laboratory carcinogenicity bioassays. Mummichog are amenable to laboratory culture and experimental manipulation (46–48). They are easily maintained in static aquaria and spawn readily in captivity, providing investigators with a year-round source of experimental animals (49).

The establishment of a positive association between PAH exposure and liver neoplasms in Elizabeth River mummichog does not constitute a proven direct cause and effect relationship and should be interpreted cautiously. These fish have been chronically exposed to an undefined, complex chemical mixture that besides containing PAHs probably includes heterocyclic and phenolic compounds, chlorinated hydrocarbons, and heavy metals. Establishment of a direct cause and effect relationship will consequently require controlled laboratory exposure studies, some of which are currently being conducted at the Virginia Institute of Marine Science.

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